

Forensic Utility of Y-STR Profiling in the Interpretation of Mixed DNA Profiles

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Abstract

Y-chromosomal short tandem repeat (Y-STR) profiling is a valuable complementary technique in forensic genetics that can be used in the interpretation of mixed biological material with significant male component and dominant female component. Y-STR forensic utility of mixed DNA profiles is reviewed and focus is placed on applications, evidential value, limitations and future developments. In the literature, Y-STR analysis was described to be selective towards male DNA and can enhance the identification and characterization of male contributors from highly unbalanced mixtures, such as sexual-assault evidence. It could also help determine multiple male contributors, paternal lines, degraded or low template male DNA and unidentified male donors. However, Y-STR profiles do not represent the identity of an individual, and paternity relatives can have the same or similar profiles. Allele dropout, stutter, peak imbalance, number of contributors, linkage between loci, population structure, and database selection are other factors that complicate interpretation. Therefore, Y-STR results have to be used in conjunction with autosomal STR results and suitable statistical methods. The prospect of discrimination and complex-mixture interpretation can be enhanced through rapidly mutating Y-STRs, massively parallel sequencing, Y-SNPs, and single-cell analysis as well as computational methods. Y-STR profiling is generally still a useful tool in cases of challenging forensic DNA evidence.

Keywords: Y-STR profiling, Mixed DNA profiles, Forensic genetics, Male–female mixtures, Paternal lineage, DNA mixture interpretation, Forensic identification

Introduction

Y-chromosomal short tandem repeat (Y-STR) Profiling is an emerging technology in forensic genetics as a valuable complementary tool when the focus is the search for male DNA in a biological sample that may consist of significant female DNA (Huffman et al., 2023). Y-STR analysis is different from autosomal STR analysis which is used to assess DNA of both parents as it focuses on polymorphic STRs located on the male-specific portion of the Y chromosome. Y-STR profiling is particularly useful for sexual-assault samples and other mixed biological samples where the male DNA can be obscured by the female DNA (Cho et al., 2023). Forensic Y-chromosome analysis as a means of characterising male contributors, identifying paternal lineages and delivering investigative information when conventional autosomal analysis is less informative is the focus of the reviews (Ge et al., 2023).

The Y is passed down the father's line with male children inheriting their Y from their father. The vast majority of the Y chromosome does not recombine regularly with the X chromosome, so that markers on the Y are passed on together as a paternal haplotype (Syndercombe Court, 2021). Therefore, the Y-STR profiles can be correlated between two males sharing the same paternal lineage. This is a desirable forensic property as well as a drawback because it makes it easy to trace the paternal line of descent but it does not allow for the identification of a unique individual. The International Society of Forensic Genetics (ISFG) thus recommends taking into consideration the paternal relatedness and dependencies between the Y-STR loci when assessing evidential weight (Roewer et al., 2020).

The Y-STRs are polymorphic repetitive DNA sequences in which polymorphisms are mainly due to the variation in the number of repeats. Y-STR haplotype is a combination of multiple loci to enhance the discriminatory power of analysis (de Knijff, 2022).

Male specificity is the main forensic value of their study. Amplification of Y-chromosomal targets will mask the male component of the DNA due to the females' large autosomal contribution, but in a male–female mixture, the male targets will be preferentially amplified, minimizing the masking effect. Review of forensic genetic markers specifically mentions that Y-STRs can be helpful if the DNA mixture is unbalanced and a minor DNA contributor is masked by the dominant DNA contributor.(**Forouzesh et al., 2022**)

This has been extended by the evolution of rapidly changing Y-STRs. The original 13-marker rapid mutating Y-STR was used on 8 individuals with 3 shared haplotypes while the 17-marker Yfiler set was used with 85 individuals, with 33 shared haplotypes. In a worldwide data set of 604 unrelated males, the 13-marker rapidly mutating Y-STR panel had only three shared haplotypes among eight individuals, while the 17-marker Yfiler panel had 33 shared haplotypes among 85 individuals.(**Ballantyne et al., 2012**)

The alleles at multiple independently inherited loci are used together to give the autosomal STRs a greater individual discrimination. But when there is a large difference in a mixed sample between male and female contributors, their interpretation can be tricky. For some problems, Y-STRs offer improvements by virtue of being passed down through the male line(**Forouzesh et al., 2022**). On the other hand, since the Y-STR loci are inherited as a linked paternal haplotype that is transmitted by the father, unrelated males can have the same profile and relatives by the male line can have identical or very similar profiles. Therefore, it is our opinion that Y-STRs should be used in conjunction with the autosomal STR typing, and not as a replacement for it.(**Ralf et al., 2024**)

Y-STR typing is primarily used in forensic science to extract useful male genetic material from male samples in which there is also female DNA. It can help to exclude or identify male contributors, to identify many male contributors or to determine the paternity line relationships. An analytical validation study of a 13 marker RM-Yplex assay successfully profiled male–female mixtures at the ratios tested and achieved complete profiles down to 62.5 pg of male

template DNA, showing the analytical sensitivity of specialized Y-STR assays(**Alghafri et al., 2015**). Y-STR profiling offers a specific technique for obtaining male-specific genetic information and thus has a significant forensic value in the interpretation of overlapping mixed DNA samples and in the consideration of the quality of the evidence.

Aim

To evaluate the forensic utility of Y-STR profiling in the interpretation of mixed DNA profiles, with particular emphasis on male–female mixtures, complex mixtures, statistical evaluation, and its limitations.

Objectives

- To examine the application of Y-STR profiling for detecting and characterizing male DNA in mixed and low-level biological evidence.
- To evaluate the advantages and limitations of Y-STR profiling in comparison with conventional autosomal STR analysis.
- To assess emerging developments, including rapidly mutating Y-STRs, massively parallel sequencing, and computational approaches, for improving the interpretation of complex forensic DNA mixtures.

Research Method

The method of this research was descriptive and critical review, which consist of an evaluation of the forensic utility of Y-STR profiling in the interpretation of mixed DNA profile. Relevant literature on the use of Y-STR, mixed biological evidence, male–female mixtures, identification of the paternal lineage, statistical analysis, and new forensic technologies was reviewed. The biological properties of Y-STRs, their male-specific amplification, their use in sexual-assault and other mixed-DNA cases, and issues surrounding complex male mixtures were all discussed in the review. Specific emphasis was placed on allele dropout, stutter, peak imbalance, contributor number, paternal relatedness, haplotype frequency, population structure, and the likelihood-based interpretation. Empirical data on highly imbalanced mixtures, degraded or low template DNA,

expanded Y-STR panels and markers that undergo rapid mutation were also assessed. The evidence was comparatively summarized to compare the strengths and weaknesses of Y-STR profiling to autosomal-STR analysis. In the context of the potential future application of these methods in forensics, emerging approaches like massively parallel sequencing, Y-SNP analysis, single-cell methods, and computational mixture interpretation were discussed.

Data Source

Data for this review were gathered from secondary sources such as: peer-reviewed research publications, forensic genetics literature, scientific reviews, empirical investigations, international forensic guidelines and pertinent population databases. The studied literature was focused on Y-STR profiling, interpretation of mixed DNA profiles, male-female DNA mixes, paternal lineage analysis, statistical evaluation of Y-STR evidence, and new forensic technology. Published investigations on extremely imbalanced mixes, low-template and degraded DNA, quickly evolving Y-STRs, massively parallel sequencing, and computational techniques were also evaluated. In an effort to analyse the statistical and evidentiary weight of Y-STR profiles, relevant guidelines of the International Society for Forensic Genetics (ISFG), and information concerning Y-STR population databases were combined. A thorough comparison and synthesis of the selected sources was made in an effort to evaluate the forensic uses, benefits, limits and future potential of Y-STR profiling in complicated mixed DNA evidence.

Mixed DNA Profiles in Forensic Investigations

Mixed DNA profiles occur when the DNA obtained from a crime scene object is from two or more persons. They are especially relevant in sexual-assault, homicide, and assault and contact-DNA cases, where DNA can be passed from one person to another directly or indirectly. Single-source profiles are easier to interpret than multi-source profiles because there is no overlap in the alleles of different sources, no masking and no failure to amplify alleles. Contemporary reviews show contributor number,

quantity imbalance of DNA and stochastic effects and artefacts produced during the PCR process as important factors that influence interpretation. (HU et al., 2014), (Butler et al., 2024)

Nature and Sources of Mixed Biological Evidence

Mixed biological evidence includes any and all genetic material from blood, semen, saliva, epithelial cells and/or other biological material from more than one person. In forensic casework, mixtures can be the result of direct contact between a person or deposition of biological material at various times. Sexual assault evidence is especially significant as it can include the victim's and one or more male contributor's DNA. The mixed profiles can also be found in shared objects, in clothes, in weapons and in surfaces that are often handled. (Woollacott et al., 2025)

The more the number of contributors and the variation in amount and kind of their DNA, the more complex the mixture is. A major contributor can have high peaks of alleles while a minor contributor can have low levels of DNA. This means that alleles from the minor profile may be masked by the major profile. Forensic mixture interpretation problems mentioned in reviews include dropout, drop-in, stutter and amplification imbalance. (HU et al., 2014)

Male–Female DNA Mixtures

The most significant mixture types that may be encountered in forensic genetics include male–female mixtures, especially in sexual assault cases. The female DNA may be a significantly higher percentage of the total DNA in these samples than the male DNA (Cho et al., 2023). In normal autosomal STR analysis, DNA from both contributors will be amplified, which could mean that the DNA from the female contributor could dominate the electropherogram and hide alleles from the male contributor.

This imbalance presents a significant analysis challenge: absence of a male allele does not indicate the absence of the allele in the population. It could have been hidden behind the more numerous females or have been lost due to random fluctuations. A review of the Y-STR, DIP-STR and SNP-STR approaches specifically mentions that conventional

markers might have poor success identifying a contributor when there is a difference in the relative abundance of the two DNA sources of perhaps 10–20 times.(Forouzesh et al., 2022)

Challenges in Interpreting Complex DNA Mixtures

The more the contributors the more difficult it will be to interpret. The number of contributors, the identification of true alleles vs artefacts, contributor proportions, and the participation of alleles sharing must be determined by the analyst. In addition to this, low-template DNA may cause stochastic effects like allele dropout and peak-height imbalance. Therefore, it may be difficult to recreate full individual genotypes in complex mixtures.

In the modern interpretation, quantitative data on the height of peaks and statistical approaches that are probabilistic in nature are also utilized, in addition to visual examination. Competing propositions about contributors can be evaluated in the light of both Bayesian and likelihood-ratio frameworks, which take into account uncertainty(Mortera, 2020). The recommendations for autosomal STR and Y-chromosome STR interpretation contained in the recommendations are also separate, reflecting the need for a standardized approach to complex evidence, in line with current SWGDAM guidance.

Limitations of Autosomal STR Analysis in Male–Female Mixtures

Although the autosomal STRs are still the major markers used for forensic identification, due to their high discriminatory power, they have certain drawbacks in male–female mixtures with a large difference in numbers. Amplifying male and female DNA simultaneously will mean that if there are more female alleles than male alleles then the male alleles will be less obvious. This is especially an issue if there is a poor or low male contribution.(Forouzesh et al., 2022)

This means that autosomal STR typing can be a partial or incomplete representation of the male contributor. The fact that it is limited is what makes Y-STR profiling valuable in the forensic context as it focuses on male-specific DNA and can identify the

male part of DNA when female DNA dominates(Huffman et al., 2023).Therefore, the Y-STR analysis is not an alternative to autosomal STR analysis, but is complementary to it. Most importantly, it's also inherited through the paternal line, so a matching Y-STR profile could be a paternal lineage and not a one-on-one match for an individual(Ralf et al., 2024).Thus the interpretation of mixed profiles requires taking into account the biological features of the markers as well as the statistical model employed to analyse the evidence.(Forouzesh et al., 2022)

Forensic Application of Y-STR Profiling to Mixed DNA Evidence.

Y-chromosomal short tandem repeat (Y-STR) profiling is especially useful when both male and female DNA are found in a forensic sample and the male DNA component is not easily separated by traditional autosomal STRs. Y-STR analysis can recover male genetic information when the female input is predominately large because a portion of the Y chromosome is unique to males. The International Society for Forensic Genetics (ISFG) has determined that analysis of the male component in complex mixtures is one of the major areas of use for Y-STR testing. The technique is therefore especially useful in cases of sexual assaults, contact prints, missing or degraded specimens and unidentified male donors.

Forensic Application of Y-STR Profiling to Mixed DNA Evidence

Compared to microsatellite markers, the biggest benefit of Y-STR profiling in mixed evidence is that it can be used to selectively amplify male-derived DNA without the need to physically separate male and female cells. Conventional autosomal STR testing is performed on both sexes and can fail to detect a low level male contribution if there is a high-level female component. With Y-STR amplification this will be mitigated since the females are less likely to have the targets they are examining on the Y-chromosome.(Roewer et al., 2020)

This selectivity may be significant in very unbalanced mixtures. However, it is reported that male Y-STR profiles can be identified when a female-profile is present as much as 100,000 times more than the male.

This does not mean that all such samples will provide a full profile, as the quality of the DNA and the presence of inhibitors, degradation and stochastic amplification are important. However, it shows the significant advantage of analytical targeting of males.(Gov.uk, 2025)

Y-STR can additionally offer proof of numerous male contributors. If a man has more than one father, then, with a few exceptions, the presence of alleles or haplotypes not matching at more than one Y-STR locus could suggest a male–male mixture, but would need to be viewed in conjunction with allele sharing and mutation.(Huffman et al., 2023)

One of the most significant uses of Y-STR profiling is in sexual-assault investigation cases. Victim DNA can be present in large quantity and male DNA in small quantity in vaginal, anal or oral swabs. In cases where the male contributor is not present in the autosomal profile or is under-represented, then Y-STR testing can be used in conjunction with the autosomal profile. The ISFG suggestions explicitly specify vaginal specimens which may comprise semen with no identifiable sperm cells as circumstances where the use of Y-STR may be especially suitable.(Roewer et al., 2020)

This method also works in cases of azoospermia, vasectomy or non-ejaculatory sexual contact, where the lack of sperm does not mean that there is no male DNA. In addition to sexual-assault investigations, Y-STRs could be used to investigate mixtures of blood, objects that have been touched, clothing, fingernails, and other physical evidence specimens that contain male DNA as a minor component.(Kayser, 2017)

Y-STR Profiling in Highly Degraded or Low-Level Male DNA

Environmental degradation is a significant problem due to the fragmentation of DNA to shorter pieces after exposure and less successful amplification of the longer STR targets. Thus, reduced amplicon or “mini-Y-STR” systems have been the subject of Y-STR research. Park et al. created multiplexes that amplify

21 Y-STRs, including short amplicons, and found that template DNA concentrations of 30 pg male DNA could be reliably amplified. They also found that their experiments yielded better results for degraded DNA than a traditional Yfiler method.(Park et al., 2007)

Adaptation of Y-STR analysis to degradation has been shown to be crucial in more recent studies. A 2026 study revealed that Y-STR and autosomal STR allele-detection rates were also impacted by DNA degradation in different ways and that the degradation pattern itself could impact on the recovery of alleles. Therefore, DNA quantity and degradation should be taken into account in addition to concentration in the analysis of low-template Y-STRs.(Nakao et al., 2026)

Identification of Paternal Lineages in Forensic Investigations

Y-STR profiles are inherited primarily through the male line, which means that they do not identify any particular man but represent a male lineage. This can be helpful if a direct reference sample from the person concerned is not available, and can be used to establish an association with a specific family line of origin in males. Y-STR profiles can also be utilized in missing persons, unidentified remains, historical and kinship cases.(Kayser, 2017)

Using larger panels of Y-STRs and fast mutating markers can make the discriminatory capability much greater. For instance, one of the recent studies published in Scientific Reports in 2022 was able to extend the population data of Ibiza from 12 to 23 Y-STRs, which increased the discrimination capacity from 0.5952 to 0.9048 and the haplotype diversity from 0.9808 to 0.9977(Navarro-López et al., 2022). It should be noted, though, that paternal relatives may have very similar or even identical profiles, and that a Y-STR match would not necessarily be a one-person match. ISFG guidelines stress that Y-STR loci must not be used as independently inherited markers, and that they must be evaluated statistically using population and/or lineage-based methods.(Roewer et al., 2020)

Table 1: Selected empirical findings demonstrating the forensic utility of Y-STR profiling.

Application/Study	Y-STR approach	Reported finding	Forensic significance	Reference
Detection in highly	Male-specific	Male profile	Demonstrates strong	(Gov.uk,

unbalanced mixtures	Y-STR profiling	reported as detectable with >100,000-fold female DNA excess	male-specific selectivity in male–female mixtures	2025)
Low-template/degraded DNA	21-locus Y-miniplexes	Reliable results reported down to 30 pg male DNA	Supports analysis of highly limited or degraded male DNA	(Park et al., 2007)
Expansion of Y-STR panel	23-marker multiplex	Discrimination capacity increased 0.5952 → 0.9048	Demonstrates increased differentiation with additional markers	(Navarro-López et al., 2022)
Relative-male differentiation	50 Y-STR markers	56.2% of tested related male pairs were separated	Shows potential of expanded/RM-Y-STR panels for distinguishing paternal relatives	(Ye et al., 2022)

Interpretation of Y-STR Profiles in Complex Mixtures

The number of male contributors, relative contribution of each male, allele sharing and technical artefacts need to be assessed in the interpretation of Y-STR mixtures. Y-STR analysis is useful for male–female mixtures, as it targets only the DNA of males, however mixtures of two or more males are problematic, since the male contributors share the same class of markers and the loci of the Y-STRs are inherited as linked haplotypes. This means that the standard practice of assuming independence of loci typical of autosomal STRs cannot be used for interpretation. The authors of the paper pointed out that the use of mixture interpretation in Y-STR is unique and should be handled differently when it comes to probability of exclusion and likelihood-ratio (LR) calculations.**(Ge et al., 2010)**

Identification of Major and Minor Male Contributors

A mixed Y-STR profile may have one or more weak contributors along with a relatively strong contributor of the male side. Variations in the peak height or intensity of the signal at the individual loci can help help determine if one male has contributed a large amount of DNA to the mixture, or if it has contributed less. The significance of peak height is, however, not to be judged alone for unequal signal due to amplification variability, degradation and stochastic

effects may occur in single-source samples.**(Huffman et al., 2023), (Gov.uk, 2025)**

If a clear major Y-STR component has been identified, a profile may be interpreted in a similar manner to a single-source profile; however, when a profile is a Y-STR mixture, the entire electropherogram must be considered along with laboratory validation for an interpretation. Forensic advice to date has concluded that a clear major component of a mixed Y-STR profile may be considered as a single-source profile under certain circumstances.**(Gov.uk, 2025)**

This is particularly crucial when assessing a minor contributor profile, whose profile matches only the minor contributor. Minor alleles may be masked during analysis, or may not amplify, leading to uncertainty. Studies of the general STR mixtures have shown that the presence of a low-level contributor with a much larger contributor can make mixture interpretation difficult.**(Bille et al., 2019)**

Detection of Multiple Male Contributors

The ability to detect multiple male contributors is an important forensic application of Y-STR analysis. Where more than one allele is observed at single-copy Y-STR loci, this may suggest there is more than one male contributor. However, this criterion should be used carefully as some of the Y chromosome regions may be duplicated. For instance, the normal male profile for DYS385a/b is a constitutively duplicated, multi-copy Y-STR locus. Butler et al. showed that the duplicated loci almost always yield alleles that are

one repeat unit apart with fairly even peak heights, while the genuine male–male mixtures yield additional alleles at multiple, nonadjacent loci.

The more contributors the more complex it becomes. Using Y-chromosomal haplotype data, simulation work showed that the exclusion probability for two contributors was about 95% while five contributors reduced the exclusion probability to 70%. This is an example of the reason why even a large number of successful typing of Y-STR can leave a significant amount of uncertainty with highly complex male mixtures.(Wolf et al., 2005)

Allelic Sharing and Interpretation of Y-STR Haplotypes

Y-STR mixing is important because males from the same Y paternal line can have identical or similar Y-STR haplotypes. Thus, if a suspect's Y-STR haplotype is present in a mixture, it can be concluded that the suspect is not excluded, but not necessarily that he was the only male contributor to the mixture.

Linkage between the Y-STR loci must also be taken into consideration for the statistical interpretation. The ISFG suggests that haplotype-frequency estimation should take into account the dependence structure between markers and Y-STR loci should not be considered independently inherited variables(Roewer et al., 2020). Ge, Budowle and Chakraborty also pointed out that the mixture interpretation may involve searching reference databases for the possible haplotype of the contributors, but the greater the number of contributors and alleles, the more the calculation will be time consuming.(Ge et al., 2010)

Challenges Associated with Stutter, Dropout and Peak Imbalance

Stutter:Stutter is a PCR artefact in which the peak is typically found one repeat unit shorter than the parent allele. It is difficult to tell if the signal from a male–male Y-STR mixture is an allele or a stutter peak, if a real minor-contributor allele is present at that position. It has been demonstrated in studies that the rate of Y-STR stutter is dependent upon not only the allele, but also the height of the parental peak, highlighting the need for evaluation of stutter rates at specific loci and alleles.(Andersen et al., 2011)

Allele dropout: Another important issue is allele dropout, which tends to be a problem when one of the contributors has a very small quantity of DNA. A true allele might not contribute to the peak and may result in a partial representation of the haplotype of the given contributor. The more the DNA the less the stochastic effects.(Bieber et al., 2016)

Peak imbalance:Peak imbalance may make interpretation more complicated. Different peak heights can be due to differences in proportions of the contributors, degradation or variation in the amplification of the locus. Therefore, it is important for analysts to view the profile as a whole and not just individual peaks. In current Y-STR interpretation protocol, the number of contributors is discussed in conjunction with the position and peak-height imbalance of the stutter to evaluate possible mixtures.

Statistical and Evidential Evaluation of Y-STR Profiles

The statistical evaluation is crucial to assigning the amount of evidential significance to a Y-STR profile generated from forensic evidence. The second difference between autosomal STRs and Y-STRs is that the latter are not treated as independent markers but are inherited together as a paternal haplotype. In evaluating evidential weight, haplotype frequencies, database composition and population structure and paternal relatedness should be taken into account in the interpretation of Y-STRs, according to the International Society for Forensic Genetics (ISFG).(Roewer et al., 2020)

Y-STR Haplotype Frequency and Population Databases

Forensic evaluation is focused on the frequency of a Y-STR haplotype in an appropriate reference population. This is because the individual Y-STR allele frequencies should not be multiplied together, due to the fact that the markers are linked on the non-recombining Y chromosome. Rather, the entire haplotype is considered the statistic unit and the frequency of the haplotype is determined from actual population data or an appropriate evolutionary model.(Roewer et al., 2020)

Population databases, like Y Chromosome Haplotype Reference Database (YHRD), provide reference information to estimate haplotype frequencies and quantify population variation. Population research in India is a good example of the significance of geographically and genetically suitable databases. Shrivastava et al. have found 147 haplotypes for 173 males from central India, comprising 125 unique haplotypes and a haplotype diversity of 0.9979. This indicates the need to use population-specific datasets when assessing the rarity of a forensic haplotype.(Shrivastava et al., 2016)

Haplotype Match Probability and Evidential Weight

The value of a Y-STR match is not based upon the match but rather the frequency of the matching haplotype in an appropriate reference population. Recent research demonstrated, for samples of 17 Y-STRs and at least 1000 males per database, that the discrete Laplace method resulted in likelihood ratios $\geq 10\,000$ in 94% of cases, compared to median likelihood ratios based on the kappa method. The study also found that 50% of matches of Yfiler Plus were found within 5 meioses, suggesting the paternal relatedness in evidence weight. Therefore, a Y-STR match should be reported as a level of confidence in the match in statistical terms and as part of the lineage context, not as a stand-alone individual identification.(Andersen et al., 2023)

Likelihood-Based Interpretation of Mixed Y-STR Profiles

Likelihood ratios offer a useful way to compare different hypotheses concerning the involvement of a person in a mixed Y-STR profile. The recent research has focused on the haplotype-based likelihood approaches, due to the linkage of these Y-STRs and the impossibility of considering them as independent markers. This can be done with population frequencies, possible contributors and paternal relationships(Taylor et al., 2018). In more complicated cases, the past few years have also seen the development of computational and single-cell methods due to the fact that the traditional interpretation technique is still challenging in the

presence of multiple males in a sample.(Huffman et al., 2023)

Role of Population Substructure and Relatedness

The frequency of a Y-STR haplotype and evidential weight may be affected by the population composition. This is especially true in strongly-structured populations where the male line is more dominant. A recently published 2026 population structure study showed that the choice of database may impact estimated haplotypes based on Y-STRs and, therefore, the weight given to Y-STR evidence(Dooley et al., 2026). There is also a significant regional variation in the Y-STRs, which is reflected in the Indian population studies as well; a recent study of 23 Y-STR markers of 511 unrelated males from Madhya Pradesh showed that the population was quite diverse and differentiated, and so the use of appropriate regional reference Y-STR is advisable(Shrivastava et al., 2022). Thus, evaluation of Y-STR evidence needs to consider both population specific frequency and paternal relatedness, especially if a suspect's male-line relatives may be alternative contributors.

Comparative Forensic Utility and Limitations of Y-STR Profiling

Y-STR profiling is an important complement to forensic DNA analysis. Its primary benefit is the selective detection of male DNA, in male/female mixtures where the male DNA is in low proportion. But the Y chromosome is haploid and mainly passed through the father, so Y-STRs illustrate mostly the line of descent of individuals rather than their unique identity. Their usefulness in mixed samples has been confirmed by recent research; there is a need for better discrimination of closely related males.(Ndomondo et al., 2025)

Y-STR versus Autosomal STR Profiling

Normally, the autosomal STRs are more individual discriminating as they examine DNA information from both parents. The male component is specifically targeted with Y-STRs and they are therefore more useful in a situation where female DNA is the predominant component in a biological mixture. Forensic studies over the last few years are ongoing in

determining the value of Y-STRs for sexual-assault and other mixed-biological evidence. In addition, Huffman et al. (2023) showed the ability of single cell analysis to resolve mixtures of complex Y-STRs into individual male contributors.(Ndomondo et al., 2025)

Advantages of Y-STR Analysis in Male-Female Mixtures

The major benefit of Y-STR profiling is that it is male specific. It can supply a male genetic profile without the need for thorough separation of male and female DNA. It is especially valuable in the case of sexual assault evidence and low level male DNA or samples where the male DNA is more abundant than the female DNA. Recent reviews also identify uses in paternal-lineage and disaster victim identification.(Lee et al., 2023), (Ge et al., 2023)

Thus, Y-STR typing can be used in combination with autosomal STRs rather than in place of them. The results may contribute to determination of the presence of a male contributor and may help to identify further lineage data when standard autosomes are not available.

Limitations of Haploid and Paternally Inherited Markers

Another important limitation is the haploid and paternal inheritance of the Y chromosome. Because of the lack of independent recombination, multiple Y-STR loci are not independent markers and therefore cannot be used in isolation. The males in the same paternal lineage can have absolutely identical or very close to identical haplotypes.

Therefore, Y-STRs do not by themselves prove that the sequenced man is the sole source of the DNA. Its evidential value should only be considered taking into account haplotype frequency and potential paternal relatedness together with population structure. Recent studies have reaffirmed that there is a need for awareness of the effects of differences in population on the interpretation and discrimination of Y-STR profiles.

Challenges in Distinguishing Related Male Individuals

One of the biggest challenges with traditional Y-STR profiling is the inability to distinguish between paternal cousins. Recent developments have thus been aimed at rapidly mutating Y-STRs (RM-Y-STRs), which are able to accumulate mutations faster, and are expected to result in a higher degree of differentiation among males from the same paternal line.

In 2026, a study was conducted that identified 14 other Y-STRs that had higher mutation rates. A 44-marker panel correctly identified 46.9%, 80.4%, 86.4% and 84.8% of close relatives separated by 1–4 meioses, respectively, when used in combination with previously-developed markers. The differentiating ability estimated was 50.2%, 75.2%, 87.6% and 93.8% for the corresponding subjects. The results show very good results compared with the traditional Y-STRs, but do not discriminate between some closely related males.(Zandstra et al., 2026)

A 2026 review also shows that RM-Y-STRs are widely used for male-relative differentiation and complex forensic samples and that the differences of mutation rates between populations is an important factor to consider when interpreting results.(Ndomondo et al., 2025)

Emerging Developments and Future Forensic Applications

New advances in the analysis of the Y-chromosome are designed to address some of the constraints of traditional Y-STR analysis, such as the difficulty of distinguishing two closely related males and interpreting mixtures. The potential of Y-STR analysis in difficult forensic cases continues to grow with rapidly mutating Y-STRs, massively parallel sequencing and computational approaches.(Ndomondo et al., 2025)

The rapid mutation of the Y-STRs (RM-Y-STRs) is a significant breakthrough for distinguishing between males sharing a paternal lineage. The higher rate of mutation between paternal relatives yields more variation than traditional Y-STR markers. In 2026, 14 additional rapidly mutating Y-STRs were identified and a new, expanded panel of 44 STRs was created.

The estimated differentiation rate for relatives separated 1-4 meioses was 50.2%, 75.2%, 87.6% and 93.8% respectively. (Zandstra et al., 2026)

The results suggest that RM-Y-STRs have the potential to be a significant aid in resolving related male paternity, but cannot completely solve all the paternity problems between related males. The reliance on them in the future will rely on forensic validation, population-sensitive mutation information and established systems of interpretation.

Massively parallel sequencing (MPS/NGS) is enhancing the resolution of Y-STR analysis by adding sequence data to the traditional allelic-length data. This can reveal sequence differences between alleles and add to the resolution between apparently identical alleles.

In 2026, they created a 365-plex NGS panel comprised of 57 Y-STRs and 308 Y-SNPs. The panel aims to deliver short- and long-term paternal-relative information through Y-STRs, and ancestry-resolving paternal-lineage information using Y-SNPs, showing the potential of integrated sequencing-based methods for forensic identification and ancestry analysis. (Li et al., 2026)

NGS could thus be of specific interest for degraded samples, complex mixtures and cases that demand higher Y-chromosomal resolution.

One of the outstanding challenges in forensic genetics is the analysis of complex mixtures of DNA. New advances have increasingly integrated enhancements in laboratory methods with computational approaches for estimating contributor, exploring potential profiles, and estimating the strength of the evidence. The 2024 NIST scientific foundation review highlighted the following advances in modern DNA-mixture interpretation: probabilistic genotyping, massively parallel sequencing and advanced mixture models. (Butler et al., 2024)

In Y-STR mixtures, in particular, it is hoped that the computational approaches will enable separation of contributors, interpretation of shared haplotypes, and analysis of complex male mixtures to be improved. Routine use of case work for advanced computational methods requires extensive validation, however.

Future Prospects for Complex Forensic DNA Evidence

The future of Y-STRs would see the incorporation of RM-Y-STRs, NGS, Y-SNPs, single-cell analysis and computational interpretation, not just the traditional Y-STR typing. New studies show that such technologies offer more discrimination, better identification of degraded material and better differentiation of paternal relatives. (Ndomondo et al., 2025), (Li et al., 2026)

The potential forensic applications of Y-STR analysis in the future extend beyond the male lineage to multipaternal mixtures, degraded DNA, paternal relationships and hard-to-identify mixtures. However, before widespread use, factors of population variation, differences in mutation rates, sequencing artefacts and the need for forensic validation are important to consider.

Conclusion

Y-STR profiling is a useful adjunct to autosomal STR analysis in mixed biological samples, particularly when male DNA is present at low levels or is masked by a dominant female component. It has a male-specific targeting that offers significant benefits in sexual-assault cases, in degraded samples, in low-template DNA and other complex forensic cases. Y-STR profiles, however, do not identify individuals but do reflect paternal lineages and may be shared by paternal relatives. Allele dropout, stutter, peak imbalance, multiple contributors, population structure and the linked inheritance of Y-STR loci can also influence interpretation. So, use of Y-STR in conjunction with autosomal STRs and a proper haplotype-based statistical evaluation should be considered, not as a replacement. Newly identified, fast-changing Y-STRs, massively parallel sequencing, Y-SNPs, and bioinformatics are all potentially powerful resources for discrimination and interpretation of the increasingly complex forensic DNA mixtures.

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